Terfenadine Antagonism Against Interleukin-4-Modulated Gene Expression of T Cell Cytokines

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Here, we investigated whether an anti-allergy drug, terfenadine, affects interleukin-4-modulated cytokine expression in peripheral T cells. Peripheral blood T cells were first stimulated with recombinant interleukin-4 and then tested for modulation of the mRNA of a panel of cytokines using the reverse transcription–polymerase chain reaction followed by Southern blot analysis. It was found that T cells constitutively expressed mRNA specific to T helper 1 cytokines (interleukin-2, interferon-γ, tumor necrosis factor-α), which was markedly downregulated upon stimulation with interleukin-4, whereas mRNA for T helper 2 cytokines such as interleukins 4, 5, and 6 was induced in response to interleukin-4. Interestingly, the interleukin-4-induced expression of all T helper 2 cytokines examined was markedly downregulated by terfenadine. Among T helper 1 cytokines, interleukin-4-mediated suppression of tumor necrosis factor-α was not affected by terfenadine, which, however, markedly restored mRNA expression of interferon-γ or interleukin-2. Electrophoretic mobility shift assays using [^{32}P]-labeled synthetic oligonucleotides encoding the consensus binding motif of activator protein-1 demonstrated that interleukin-4-induced binding of activator protein-1 composed of JunB was interfered by terfenadine. This study indicates that terfenadine, at least partially, interferes with interleukin-4-activated signaling, leading to terfenadine antagonism against the modulatory impact of interleukin-4 on T cell cytokines. ***Key words: activator protein-1/JunB/terfenadine/T helper 1/T helper 2. J Invest Dermatol 121:490–495, 2003***

A rapid increase in allergic skin disorders has recently been observed with approximately one-third of patients attending dermatology clinics. Atopic dermatitis, in particular, has currently been highlighted due to long-lasting itchy rashes, which may affect the entire surface of the body. In respect to pathogenesis of atopic skin disorders, there is belief that T helper (Th) 2-type immune responses play a pathogenic role (Akdis *et al*, 2000). This hypothesis may be supported by the observations that: (1) elevated serum levels of IgE is frequently observed in patients (Akdis *et al*, 2000), and (2) skin-infiltrating T cells predominantly produce Th2-type cytokines such as interleukin (IL)-4 and IL-5 (Hamid *et al*, 1994). Collectively, it might be possible to postulate that atopic skin disorders are triggered by cytokine-driven signals derived from Th2 cells, such as IL-4.

Terfenadine is an anti-allergic drug, which is a nonsedating histamine H1 receptor antagonist (Woodward and Munro, 1982). In addition to its potent antihistaminic effects, it has been reported that terfenadine induces selective inhibition of Th2-type cytokine produced by human peripheral T cells *in vitro* upon stimulation with the T cell receptor and using activation of costimulatory signal via CD28 (Munakata *et al*, 1999). Besides these observations, little is known about how terfenadine affects signaling, which may be underlying those changes. To explore further the suggestion that atopic skin disorders might be mediated by Th2-type immunity, we undertook this study to explore further the effects of terfenadine on IL-4-modulated cytokine expression on peripheral T cells.

Our results show that terfenadine, at least partially, antagonizes IL-4 in the modulation of mRNA expression of T cell cytokines. In addition, terfenadine can antagonize IL-4-activated binding of transcription factor, activator protein (AP)-1 bearing a JunB component.

MATERIALS AND METHODS

Preparation of freshly isolated peripheral T cells Blood samples were obtained from healthy volunteers under a protocal approved by the Institutional Review Board of Kin Ki University School of Medicine. Accordingly, informed consent was obtained from each individual. To first isolate peripheral blood mononuclear cells, 20 to 30 mL of peripheral blood collected from healthy volunteers was layered on top of 20 mL density gradient solution (Ficoll-Paque; Amersham Pharmacia Biotech AB, Uppsala, Sweden) and centrifuged. Subsequently, peripheral blood mononuclear cells recovered from the interface were incubated with magnetic beads coupled with monoclonal antibodies directed against CD19 and CD14 (Dinabeads; both purchased from Dynal A.S., Oslo, Norway). Cells unbound to the beads were finally resuspended in RPMI1640 medium supplemented with 10% human AB serum, 1% L-glutamine and 1% antibiotics/anti-mycotics. Percent enrichment was quantitated by fluorescence-activated cell sorter analysis using a panel of antibodies (CD3, CD14, CD19), which revealed that approximately 95% of the preparations were T cells.

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Abbreviations: AP-1, activator protein-1; EMSA, electrophoretic mobility shift assay; STAT, signal transducer/activator of transcription.

Cell stimulation T cells were first incubated with 20 nM terfenadine (kindly provided by Aventis S.A., Strasbourg, France) or left untreated for 30 min, and stimulated thereafter with 10 ng per mL recombinant human IL-4 (R&D Systems, Minneapolis, Minnesota) or left unstimulated. For some experiments, T cells were stimulated with histamine ranged at 10^{-5} to Ø M. Other anti-allergic drugs, ketotifen or promethazine (both purchased from Sigma, St Louis, Missouri) were used as controls.

RNA extraction Terfenadine-pretreated cells were stimulated with 10 ng per mL recombinant IL-4 or left untreated, and harvested 3 h later. RNA was extracted according to the acidic phenol procedure described previously (Aragane *et al*, 1996). The amounts of RNA in samples were determined photometrically at 260 nm and the RNA samples were subjected to the reverse transcription–polymerase chain reaction (reverse transcription–PCR).

Reverse transcription–PCR Five micrograms of total RNA was reverse transcribed using dT17 (Takara, Tokyo, Japan). Semi-quantitative reverse transcription–PCR amplification was conducted as described elsewhere (Aragane *et al*, 1996). Briefly, we amplified all cDNA samples with primers specific to β-actin using various cycle numbers and dilution factors, and thereby determined the optimal concentration of each sample and a cycle number showing a logarithmic increase in amplification. Samples were then amplified using primers specific for IL-2, IL-4, IL-5, IL-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, respectively. All PCR primers used in this study were purchased from Clontech (Palo Alto, California).

Southern blot analysis To monitor specificity of PCR signals, Southern blot analysis was performed. Briefly, PCR products were fractionated in a 2% agarose gel and transferred on to nylon membranes (Hybond; Amersham, Buckinghamshire, UK) by employing a capillary blotting method. The membranes were then hybridized with [^{32}P]-labeled cDNA probes encoding IL-2, IL-4, IL-5, IL-6, IFN-γ, and TNF-α, respectively, in 5 × sodium citrate/chloride buffer, 5 × Denhardt's solution (0.1% polyvinylpyrrolidone, 0.1% Ficoll 400, 0.1% bovine serum albumin), 0.5% sodium dodecyl sulfate (all chemicals purchased from Sigma) at 65°C overnight. After washing in 2 × SSC for 10 min twice at room temperature, 2 × SSC/1% sodium dodecyl sulfate for 30 min twice and 0.1 × sodium citrate/chloride buffer for 30 min twice at room temperature, the membranes were autoradiographed to X-ray films at –80°C. PCR signals on the Southern blot analysis were densitometrically quantitated using a densitograph unit (Atto, Tokyo, Japan).

Nuclear protein extracts Fifteen minutes after stimulation, the cells were collected in tubes and washed three times with chilled phosphate-buffered saline by centrifugation at 200 × *g*, 4°C for 5 min. Preparation of nuclear extracts was performed essentially as described previously (Aragane *et al*, 1997). Briefly, cells were allowed to swell in a hypotonic buffer (10 mM HEPES (pH 7.8), 0.1 mM ethylenediamine tetraacetic acid, 10 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) for 15 min on ice. The samples were then mixed with Nonidet P-40 (Sigma) to a final concentration of 0.25% and minifuged at 1,850g, 4°C for 5 min. After discarding the supernatants, the pellets were resuspended in hypertonic buffer (20 mM HEPES (pH 7.9), 350 mM NaCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride), and incubated on ice for 15 min. Both fractions were stored at –80°C until being used. These samples were used for electrophoretic mobility shift assays (EMSA) as nuclear extracts.

EMSA Synthetic double-stranded oligonucleotides encoding the consensus binding motif of AP-1 or signal transducer/activator of transcription (STAT)-5 and -6 (STAT-5/6) were purchased from Santa Cruz (Santa Cruz, CA). Binding reactions were performed with 5 μg of protein, 5 × 10^4 cpm of the [^{32}P]-labeled double stranded oligonucleotides, 50 mM KCl, and 4 μg of poly(dI-dC) (Boehringer Mannheim, Mannheim, Germany) for 25 min at room temperature. To monitor specificity of the binding, nuclear protein fractions were preincubated with an antibody directed against JunB (*sc-73*, Santa Cruz), c-Jun (Ab-1, Oncogene, San Diego, CA), JunD (*sc-74K*, Santa Cruz), c-Fos (Ab-2, Oncogene), or with nonimmune rabbit serum used as an irrelevant control IgG. The anti-JunB recognizes the C-terminus of the protein, which is crucial for the binding to the specific DNA sequences (Ryder *et al*, 1988). Therefore, according to a previous report, the addition of the antibody to the reaction usually results in abrogation of the binding (Yamazaki *et al*, 2002). Reaction mixture was applied to native high ionic gels (1 × Tris/glycine buffer, 5% acrylamide (acrylamide/bis ratio, 19:1)) and electrophoresis was performed at 100 V for 90 min; then the gels were dried by a gel dryer and autoradiographed at –80°C.

Enzyme-linked immunosorbent assays (ELISA) To quantify cytokines released from T cells, supernatants were collected 18 h after stimulation and applied to ELISA specific for various cytokines, including IL-2, IL-5, IL-10, IL-13, TNF-α, and IFN-γ. All ELISA systems were purchased from R&D Systems.

RESULTS

Terfenadine suppresses histamine-induced production of Th2 cytokines, IL-5 and IL-10 Terfenadine is a nonsedating H1 receptor antagonist, and therefore should be able to block biologic events induced by histamine. Therefore, we first checked the effects of terfenadine on histamine-induced cytokine production. To do so, T cells were first preincubated with or without 20 nM terfenadine for 30 min and then stimulated with various concentrations of histamine. Supernatants were collected 18 h later and production of IL-5 and IL-10, Th2 cytokines and TNF-α, a Th1 cytokine, were tested using specific ELISA systems. The production of both IL-5 and IL-10 was significantly enhanced by histamine in a concentration-dependent manner, whereas in the presence of terfenadine histamine failed to upregulate the production (**Fig 1*a*,*b***). This indicates that histamine-induced production of Th2 cytokines was counterregulated by terfenadine through blockage of activation of histamine receptors. In contrast, histamine did not affect the constitutive production of TNF-α, which was not further modulated by terfenadine (**Fig 1*c***). It is noteworthy that in the absence of histamine terfenadine was not able to modify the constitutive production of IL-5, IL-10, or TNF-α, indicating that contamination of endogenous histamine, i.e., derived from serum or T cell preparation, if present, did not play any part in the production of T cell cytokines tested. The concentration of terfenadine used in this study (20 nM) turned out to be nontoxic for T cells as viability of T cells was not significantly affected by this concentration of terfenadine as measured by a trypan blue exclusion assay (**Fig 1*d***). Based on these observations, we used this concentration of terfenadine (20 nM) throughout this study.

Terfenadine antagonized IL-4-modulated mRNA expression of T cell cytokines As terfenadine is known to improve the clinical symptoms of atopic skin disorders, in which IL-4 plays a central part, we hypothesized that the pharmacologic effects of terfenadine are, in part attributed to its antagonism against IL-4. To address this issue, we first tested IL-4-induced modulation of Th2-type cytokines, including IL-4, IL-5, and IL-6 in T cells. Therefore, PCR amplification using primers of the respective cytokines was conducted, followed by Southern blot analysis to confirm the specificity of the PCR signals. Constitutive expression of mRNA specific for these Th2-type cytokines was markedly induced upon stimulation with IL-4 (**Fig 2*a***). The addition of 20 nM terfenadine alone to T cells did not alter the mRNA expression of cytokines tested, excluding the possibility that terfenadine itself affects the respective message in the absence of IL-4 (data not shown). Interestingly, 20 nM terfenadine markedly prevented upregulation of IL-4-induced mRNA expression for all Th2-type cytokines tested. Together, these data indicate that terfenadine suppressed IL-4-induced mRNA expression specific for Th2 cytokines in T cells. We next focused on modulation of Th1 cytokines in T cells by IL-4. As shown in **Fig 2(*a*)**, constitutive mRNA expression of Th1-specific cytokines was markedly downregulated by stimulation with IL-4. IL-4-induced downregulation of TNF-α mRNA was not affected by terfenadine, which, however, markedly restored IL-4-downregulated expression of IFN-γ (**Fig 2*a***). Furthermore, downregulated expression of IL-2 mRNA was moderately

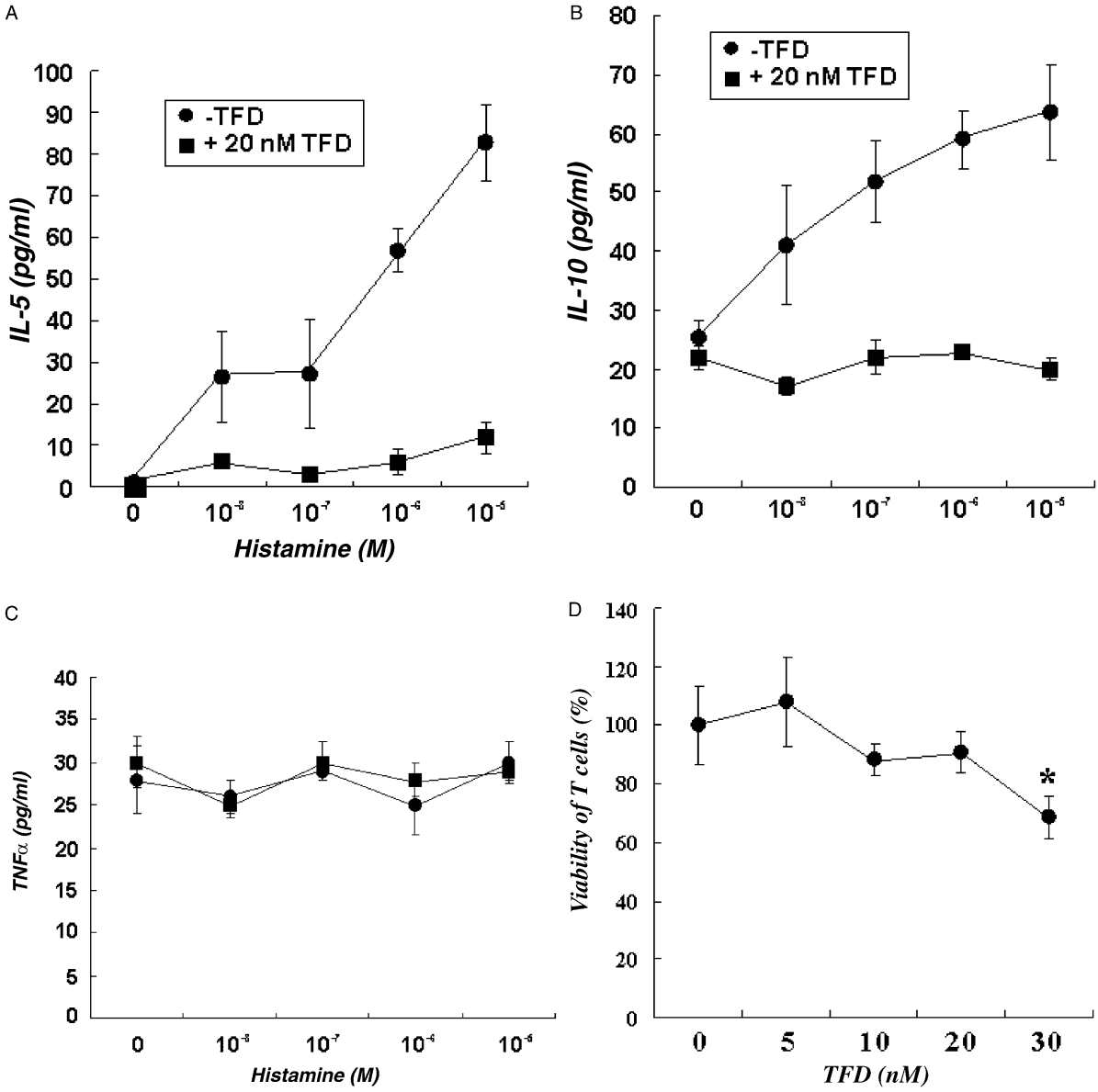

Figure 1. Terfenadine inhibited histamine-induced cytokine production from T cells. Peripheral T cells derived from healthy donors (n = 3) were cultured in the presence or the absence of 20 nM terfenadine for 30 min, and either stimulated with various concentrations of histamine (0–10^{-5} M) or left untreated. Eighteen hours later, culture supernatants were collected, and cytokines released to the supernatants were quantified using ELISA kits specific for IL-5 (*a*), IL-10 (*b*), and TNF-α (*c*). Note that this concentration of terfenadine used throughout this study was not toxic for T cells that were cultured in the presence of 20 nM terfenadine for 18 h (*d*). An asterisk in (*d*) indicates a statistically significant decrease in viability of T cells when compared with those cultured in the absence of terfenadine (0 nM). TFD; terfenadine.

restored by terfenadine (**Fig 2**). These data indicate that terfenadine, at least partially, counteracts IL-4-induced modulation of gene expression in respect to Th1 cytokines. The PCR signals were quantified densitometrically as shown in **Fig 2(*b*)**. Collectively, terfenadine tends to antagonize IL-4-modulated expression of T cell cytokines.

Modulations of IL-4-induced cytokine production by terfenadine Based on the RNA data shown in the above, we next checked modulation of IL-4-induced cytokine production by terfenadine at the protein level. Therefore, we performed ELISA assays specific for various cytokines including IL-2, IL-5, IL-10, IL-13, TNF-α, and IFN-γ (**Table I**). To examine the modulatory effects characteristic of terfenadine, other anti-allergic drugs, ketotifen and promethazine were used as controls, both of which are well known H1R antagonists. For this purpose, T cells were first preincubated with either 20 nM terfenadine, 20 nM ketotifen, 20 nM promethazine, or left unincubated. In the preliminary experiments, we added various concentrations of either drug on freshly isolated T cells and checked the viability. Viability of T cells were significantly impaired (the viability $<50\%$) when 10-fold higher concentration of each drug was added (data not shown). Thirty minutes later, cells were stimulated with 10 ng per mL IL-4 or left unstimulated, 18 h later supernatants were harvested, and ELISA assays were performed. Confirming the RNA data (**Fig 2**), ELISA assays revealed that constitutive production of Th1 cytokines (IL-2, IFN-γ, TNF-α) was suppressed by IL-4, which induced the release of Th2 cytokines, such as IL-5, IL-10, and IL-13 (**Table I**). Terfenadine downmodulated the IL-4-enhanced production of Th2 cytokines (IL-5, IL-10, IL-13), whereas it partially, but significantly restored IL-4-suppressed

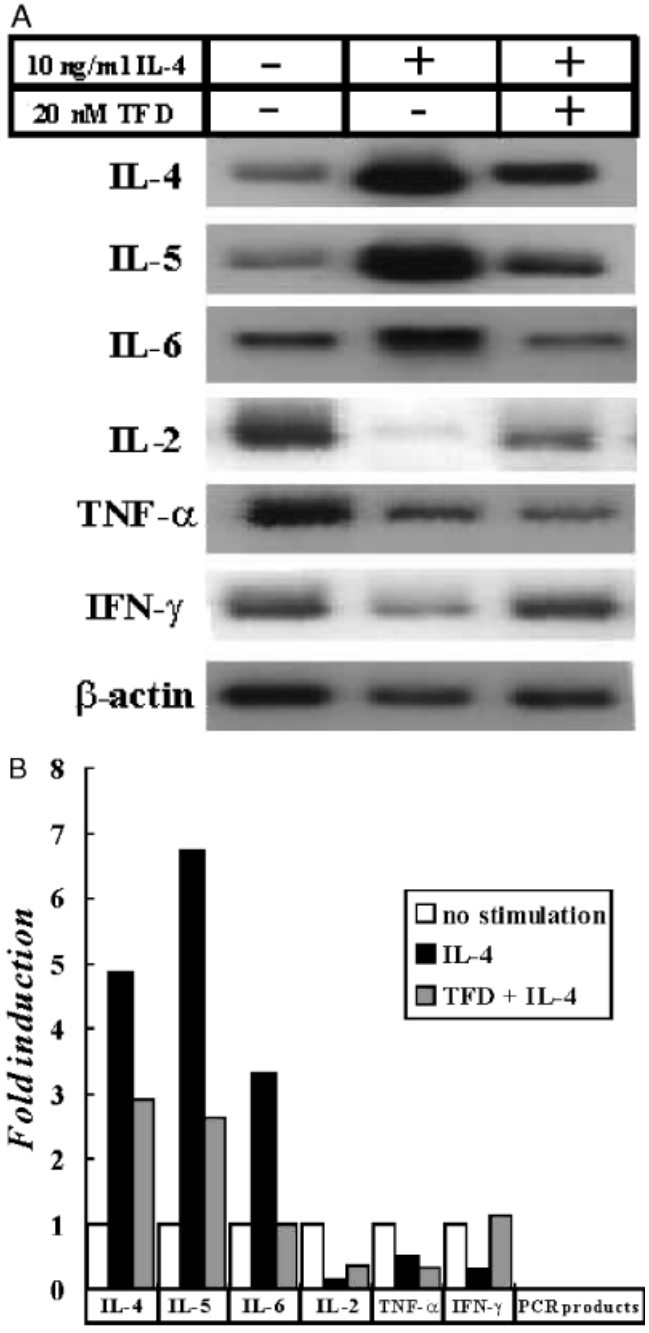

Figure 2. Terfenadine modified IL-4-induced mRNA expression of cytokines in peripheral T cells. (*a*) Peripheral T cells derived from healthy donors (n = 5) were cultured in the presence or absence of 20 nM terfenadine for 30 min, and either stimulated with 10 ng per mL recombinant IL-4 or left untreated. Three hours later, the cells were harvested, total cellular RNA was extracted and reverse transcribed, and reverse transcription–PCR analysis was performed using primers specific for Th2-type cytokines, including IL-4, IL-5, IL-6, or IL-2, TNF-α, IFN-γ (Th1 cytokines) as indicated. Subsequently, Southern blot analysis was performed as described in *Materials and Methods*. Note that the addition of 20 nM terfenadine alone did not alter the basal expression of each cytokine nor of β-actin (data not shown). (*b*) PCR signals were quantified densitometrically and the fold induction was shown. To do so, a densitometric value of each PCR signal was divided by that of β-actin at the respective time point, and that of unstimulated cells of each cytokine was then scored as 1.

production of Th1 cytokines (IL-2, TNF-α), but failed to do so for IFN-γ (**Table I**). In contrast, ketotifen exerted such modulatory effects on TNF-α and IL-13, whereas promethazine did so for TNF-α only (**Table I**). When T cells were treated with the 10-fold lower concentration (2 nM) of ketotifen and promethazine and then stimulated with 10 ng IL-4 per mL, the production of TNF-α protein was 18.4 ± 0.65 pg per mL and 16.3 ± 1.01 pg per mL, respectively, both of which were not as impressive as that observed with 20 nM of each drug (data not shown). The terfenadine effects on IL-4-downregulated IFN-γ and IL-4-downregulated-TNF-α protein release were inconsistent with those seen at their mRNA levels (**Fig 2**). Although the reason for this discrepancy is presently unknown, it might be that terfenadine is able to post-transcriptionally inhibit protein synthesis of TNF-α and the opposite is true for IFN-γ. This issue still remains unknown. Overall, however, there is also a tendency that terfenadine antagonizes IL-4-modulated production of T cell cytokines at protein levels.

Terfenadine interferes with IL-4-mediated activation of the transcription factor, AP-1 To get further insight into the effect of terfenadine on IL-4-induced modulation of T cell cytokines, we next focused on signaling events activated by IL-4. To do so, a transcription factor, AP-1, was selected, and EMSA were performed using [^{32}P]-labeled synthetic oligonucleotides encoding the consensus binding sequences for AP-1 (**Fig 3**). It was found that AP-1 binding was activated upon IL-4 stimulation, whereas in the presence of terfenadine IL-4 failed to activate AP-1 binding (**Fig 3**). Furthermore, the IL-4-induced AP-1 binding was prevented by preincubation of the nuclear extracts with an antibody directed against JunB (**Fig 4**), a component of AP-1 complex, whereas the binding was not affected by anti-c-Jun, anti-c-Fos, and anti-JunD. Together, this indicates that the AP-1 complex activated by IL-4 is composed of the JunB component. To address whether terfenadine affects IL-4-activated transcription factors other than AP-1, [^{32}P]-labeled oligonucleotides encoding consensus binding sites for STAT-5/6 were used, and EMSA performed (**Fig 5**). STAT-6 is well known to be activated by IL-4. As initially anticipated, IL-4 activated the binding of STAT-6, whereas terfenadine failed to interfere with the IL-4-activated STAT-6 binding, indicating that terfenadine did not affect the IL-4-activated binding of STAT-6.

DISCUSSION

Terfenadine is a well-tolerated anti-allergy drug, whose therapeutic spectra widely span various allergic disorders, including allergic rhinitis, bronchial asthma, urticaria, and inflammatory skin conditions, such as eczemas and atopic dermatitis (Buckley *et al*, 1985; Rafferty and Holgate, 1987; Hjorth, 1988). The results presented in this paper support our initial hypothesis of terfenadine-mediated antagonism against IL-4-modulated gene expression of T cell cytokines. Here, we demonstrated that IL-4-mediated modulation of transcripts of the T cell cytokines tested, except for TNF-α, was counteracted by terfenadine. It previously has been shown that terfenadine inhibited the production of Th2 cytokines (IL-4, IL-5), but not Th1 cytokines (IL-2, IFN-γ) from peripheral T cells, which were stimulated via activation of the T cell receptor and costimulatory signals or their equivalents (see introductory paragraphs) (Munakata *et al*, 1999). In contrast, we stimulated T cells with IL-4. Because different stimuli may lead to activation of different signaling pathways, the results collectively indicate that terfenadine not only prevents the production of IL-4, but also antagonizes the biologic effects of IL-4.

We next assessed how terfenadine antagonizes the biologic activity of IL-4. Therefore, we focused on the transcription factor, AP-1 (Liebermann *et al*, 1998). As the binding motif of AP-1 is rather ubiquitously distributed in the promoter regions of

Table I. Comparison of suppressive effects of anti-histaminics on IL-4-modulated cytokine production

	Control	IL-4[a]	IL-4 + TFD[b]	IL-4 + KET[c,d]	IL-4 + PMT[e,f]
IL-2[g]	109.3 ± 5.85	31.3 ± 0.97	59.6 ± 3.90*[,h]	29.6 ± 2.44†[,i]	38.9 ± 3.90†[,j]
IFNγ[g]	48.3 ± 6.42	18.6 ± 6.10	20.6 ± 3.21†[,h]	16.8 ± 6.10†[,i]	19.2 ± 5.05†[,j]
TNFα[g]	69.5 ± 16.6	13.5 ± 0.77	26.5 ± 0.77*[,h]	27.4 ± 1.02*[,i]	26.4 ± 0.51*[,j]
IL-5[g]	17.6 ± 1.34	110.6 ± 8.38	71.5 ± 4.61*[,h]	89.5 ± 8.87†[,i]	104.9 ± 17.98†[,j]
IL-10[g]	19.6 ± 0.60	45.8 ± 5.56	23.9 ± 0.75*[,h]	36.7 ± 2.55†[,i]	40.5 ± 2.85†[,j]
IL-13[g]	474.5 ± 37.65	906.6 ± 8.69	705.9 ± 72.41*[,h]	700.8 ± 24.6*[,i]	890.2 ± 43.44†[,j]

[a] T cells were stimulated with 10 ng/ml recombinant IL-4.
[b] T cells were preincubated for 30 minutes in the presence of 20 nM terfenadine and stimulated with IL-4.
[c] KET, ketotifen.
[d] T cells were preincubated for 30 minutes in the presence of 20 nM ketotifen and stimulated with IL-4.
[e] PMT, promethazine.
[f] T cells were preincubated for 30 minutes in the presence of 20 nM promethazine and stimulated with IL-4.
[g] Supernatants of T cells cultured for 18 hours in the indicated culture condition were applied to ELISA assays for the respective cytokines. Each value is expressed pg/ml.
[h] Paired student-*t* tests were conducted between values of the respective cytokine produced from T cells stimulated with IL-4 alone and with TFD plus IL-4.
[i] Paired student-*t* tests were conducted between values of the respective cytokine produced from T cells stimulated with IL-4 alone and with KET plus IL-4.
[j] Paired student-*t* tests were conducted between values of the respective cytokine produced from T cells stimulated with IL-4 alone and with PMT plus IL-4.
* $p < 0.05$.
† Not significant.

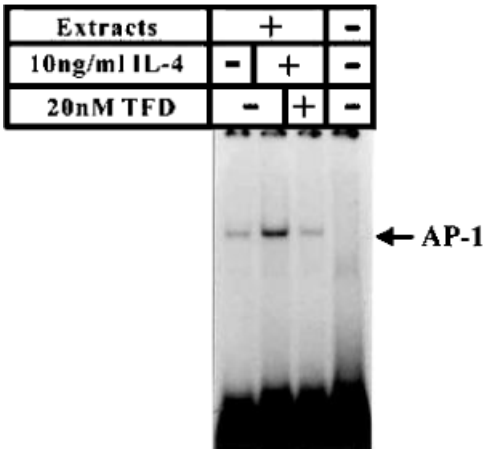

Figure 3. Electrophoretic mobility shift assays. Peripheral T cells were cultured in the presence or absence of 20 nM terfenadine for 30 min, and either stimulated with 10 ng per mL IL-4 or left untreated. Fifteen minutes later, the cells were harvested and nuclear protein fractions were prepared. EMSA was performed with 4 μg nuclear extracts using [^{32}P]-labeled synthetic oligonucleotides encoding the consensus binding sequences for AP-1. The specific binding of AP-1 is shown by the *arrow*.

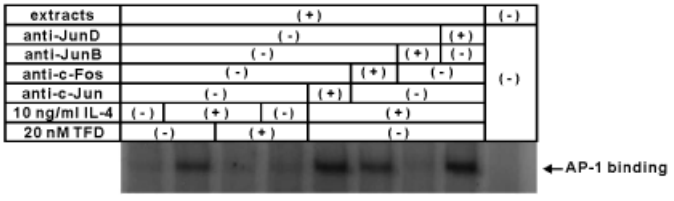

Figure 4. JunB as a component of AP-1 complex. Antibody blocking experiments were performed to characterize which nuclear proteins are activated in this experimental system. Nuclear extracts were preincubated with rabbit polyclonal antisera against JunB or an antibody directed against c-Jun, c-Fos, or JunD, followed by the identical procedures to those described in **Fig 3**.

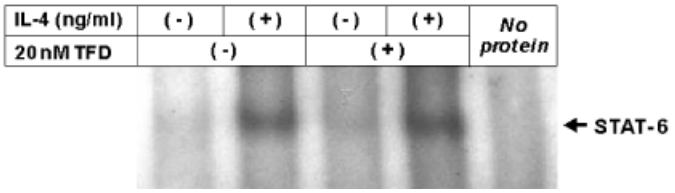

Figure 5. IL-4-activated STAT-6 was not affected by terfenadine. Peripheral T cells were cultured in the presence or the absence of 20 nM terfenadine for 30 min, and either stimulated with 10 ng per mL IL-4 or left untreated. Fifteen minutes later, the cells were harvested and nuclear protein fractions were prepared. EMSA was performed as in **Fig 4** with [^{32}P]-labeled synthetic oligonucleotides encoding the consensus binding sequences for S.

numerous genes (Dendorfer, 1996), it might be possible that activation of AP-1 leads to deregulation of biologic events, such as atopic dermatitis (Chan *et al*, 1996). Furthermore, we recently showed that the binding of AP-1 was markedly enhanced in proteins extracted from IL-4-stimulated peripheral blood mononuclear cells derived from patients with atopic dermatitis, when compared with those from healthy individuals (Yamazaki *et al* 2002). Therefore, effective antagonism of AP-1 activation may result in improvement of allergic diseases, in which overactivation of AP-1 might play a causative role. The EMSA using the consensus binding motif of AP-1 revealed that terfenadine markedly suppresses AP-1 binding induced by IL-4, suggesting that terfenadine interferes with AP-1-mediated biologic phenomena. Interestingly, IL-4-induced AP-1 binding was prevented by preincubating nuclear proteins with an antibody directed against JunB, but not with antibodies against c-Jun, c-Fos, and JunD, indicating that the IL-4-activated AP-1 complex is composed of the JunB protein. Currently, it has been reported that JunB may be involved in IL-4 expression, thereby being able to direct Th2 differentiation (Rincon *et al*, 1997; Li *et al*, 1999). In this context, our results showing the downregulation of Th1 cytokines and upregulation of Th2 cytokines, both of which are induced by IL-4, favor the concept that IL-4 dictates the fate of T cells to Th2 cells. Owing to the absence of antigen, antigen-presenting cells and/or costimulatory signals in the culture condition, we only can analyze terfenadine antagonism against IL-4-mediated signaling, but not the effect on T cell differentiation into Th2 cells. It would be interesting to check whether differentiation of Th2 cells is inhibited by terfenadine or other anti-histamines. This question is still open for further investigation.

Although suppression of IL-4-induced expression of T cell cytokines by terfenadine is clearly shown in this study, underlying mechanisms remain largely unknown. It has currently been reported that Th1 and Th2 preferentially express H1 receptor (H1R) and H2R, respectively, and that histamine through the binding to H1R induces differentiation of Th1 cells (Jutel *et al*,

2001). If terfenadine exclusively exerts the inhibitory effects through the binding to H1R in our experimental system, Th1 type cytokines should preferentially have been suppressed. Because we stimulated freshly isolated peripheral T cells with IL-4 alone and did not activate T cell receptor and costimulatory signals, T cells analyzed differed from those in Jutel *et al* (2001). Because IL-4-induced expression of Th2 cytokines, however, was clearly inhibited by terfenadine, the results imply that terfenadine through binding to molecule(s) other than H1R might affect IL-4-signaling. We presently have no firm idea how terfenadine antagonizes IL-4-induced cytokine production, i.e., activation of suppressive signals through the binding to H1R or other molecules. In this respect, it is worthy to note that anti-allergic drugs may bind to various molecules, including S100A1 and S100B, inviting the speculation that anti-allergic drugs through binding to these proteins might inhibit degranulation (Shishibori *et al*, 1999; Okada *et al*, 2002). In the respective papers, it was described that anti-allergic drugs may also bind to other molecules, such as calcineurin α and β, 14-3-3 proteins, neurocalcins and calmodulin. Although such detailed analysis of the binding proteins of terfenadine has not been performed so far, it might be possible to speculate that terfenadine through binding to surface molecule(s) may interfere with IL-4 signaling. This issue still remains to be elucidated. Furthermore, in **Table I** we have shown that ketotifen and promethazine, though less impressive than terfenadine, restored IL-4-suppressed production of TNF-α as well. Although careful titration studies of these drugs were not conducted on the effects on IL-4-modulated expression of the cytokines tested, such studies, by expanding the numbers of drugs to be tested, might bring us further insights of how anti-allergic drugs exert their immune modulatory properties.

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